DEMONSTRATED PROTOCOL

Removal of Dead Cells for Single Cell RNA Sequencing

Overview

A high percentage of non-viable cells may impact the targeted cell recovery in 10x Genomics Single Cell protocols. This Demonstrated Protocol outlines removal of dead cells from single cell suspensions. The protocol was demonstrated using peripheral blood mononuclear cells (PBMCs), dissociated tissue cells from colorectal cancer (CRC), and clear cell renal carcinoma (CCRC) patients. However, it may be used as a basis for removing dead cells from other primary cells as well as other cell lines in preparation for use in 10x Genomics Single Cell protocols.

Additional Guidance

Consult Demonstrated Protocol Cell Preparation Guide (Document CG00053) for Tips & Best Practices on handling cells and Technical Note Guidelines for Accurate Target Cell Counts using 10x Genomics Single Cell Solutions (Document CG000091) for determining accurate cell counts.

Input PBMCs for this protocol were thawed, washed, and counted as described in Demonstrated Protocol - Fresh Frozen Human Peripheral Blood Mononuclear Cells for Single Cell RNA Sequencing (Document CG00039).

Cells carry potentially hazardous pathogens. Primary tumor cells should be handled under biosafety level-2 (BSL-2) conditions. Follow material supplier recommendations and local laboratory procedures and regulations for the safe handling, storage, and disposal of biological materials.

Cell Sourcing

Dissociated tissue cells from CRC and CCRC patients were acquired from Discovery Life Sciences. The cells were thawed, washed, and counted per the Discovery Life Sciences protocol, except the steps describing the DNAse I treatment of the cells were omitted. Refer to www.dls.com for specifics.

Preparation – Buffers

Buffers Maintain at Room Temperature	Composition
1X Binding Buffer	1X Binding Buffer Stock* in Nuclease-free Water
PBS + 0.04% BSA	

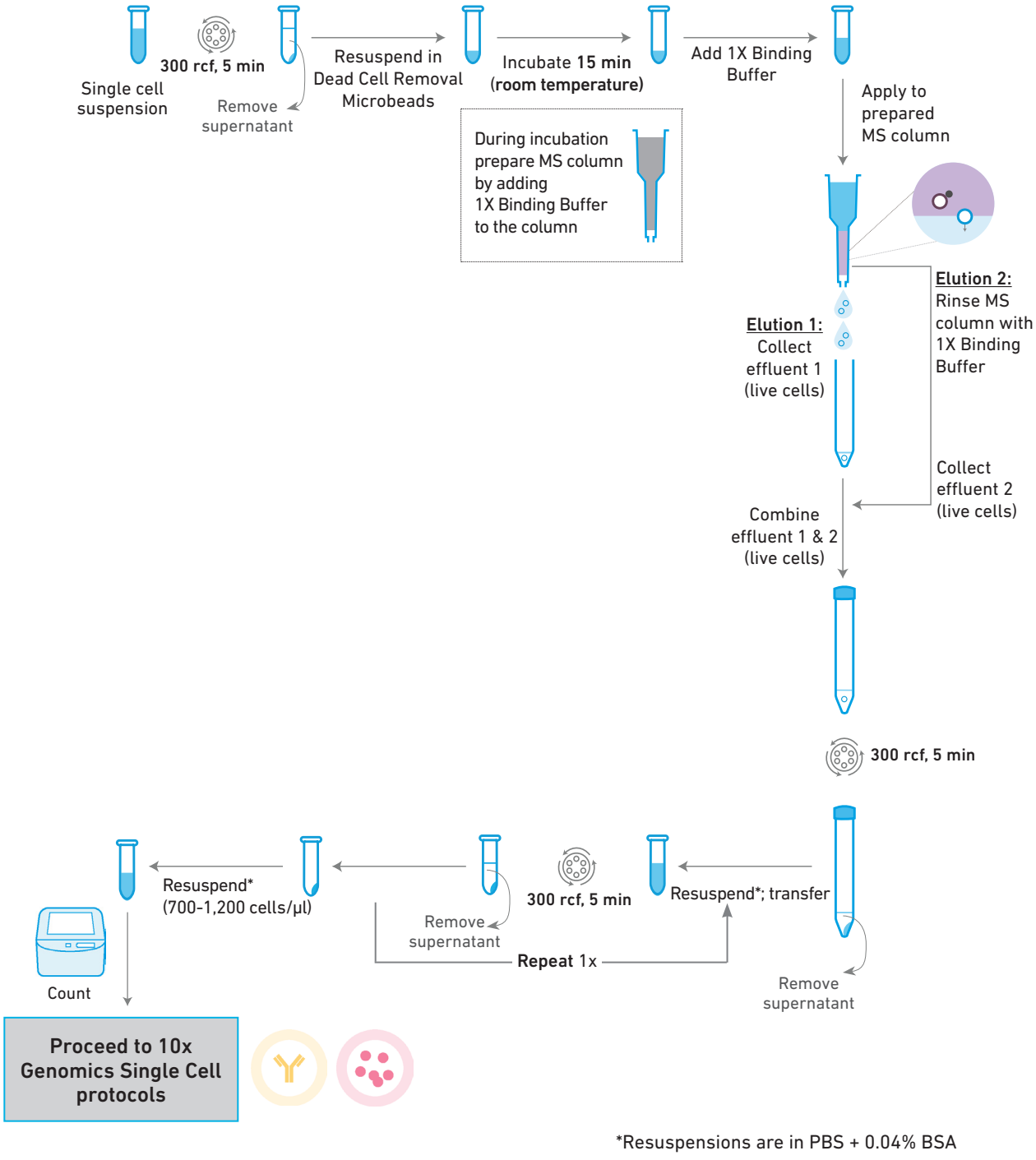
* Provided with the MACS Dead Cell Removal Kit as a 20X stock solution.

Specific Reagents & Consumables

Vendor	Item	Part Number
Thermo Fisher Scientific	Trypan Blue Stain (0.4%)	T10282
	UltraPure Bovine Serum Albumin (BSA, 50 mg/ml)	AM2616
	Countess II FL Automated Cell Counter	AMAQAF1000
	Countess II FL Automated Cell Counting Chamber Slides	C10228
	Nuclease-free Water	AM9937
Millipore-Sigma	Phosphate-Buffered Saline (PBS) with 10% Bovine Albumin (alternative to Thermo Fisher product)	SRE0036
Miltenyi	MACS Dead Cell Removal Kit	130-090-101
	MS Columns	130-042-201
	LS Columns	130-042-401
	MACS Multistand	130-042-303
	OctoMACS Separator (for use with MS Columns)	130-042-109
	QuadroMACS Separator (for use with LS Columns)	130-090-976
Bel-Art	Flowmi Cell Strainer, 40 µm	H13680-0040
Eppendorf	DNA LoBind Tubes, 2.0 ml	022431048
Corning	Phosphate-Buffered Saline (PBS) without Calcium & Magnesium	21-040-CV

Protocol Overview

Dead Cell Removal



Protocol

This protocol was demonstrated using sample sizes compatible with Miltenyi Biotech MS columns (≤2×10^8 total cells). If using sample sizes compatible with Miltenyi Biotec LS columns (≤2×10^9 total cells), consult the manufacturer's instructions.

a. Centrifuge the cell sample at **300 rcf** for **5 min** (PBMCs) or **10 min** (dissociated tumor cells).

b. Remove the supernatant without disturbing pellet.

c. Add **100 µl** Dead Cell Removal MicroBeads and resuspend cell pellet using a **wide-bore** pipette tip.

d. Incubate for **15 min** at **room temperature**.

e. During incubation, prepare MS column by rinsing with **500 µl** 1X Binding Buffer. If using the LS column, consult the manufacturer's instructions for more information.

f. After incubation is complete, dilute the cell suspension (containing Dead Cell Removal MicroBeads) with **500 µl** 1X Binding Buffer.

g. Apply cell suspension to the prepared column. The positively selected dead cells will be retained on the column while the negatively selected live cells pass through the column.

DO NOT apply the plunger supplied with the column, otherwise positively selected dead cells will be collected in the effluent.

h. Collect the effluent (effluent 1) containing the live cell fraction in a 15-ml centrifuge tube.

i. Rinse the column with **2 ml** 1X Binding Buffer and collect the effluent (effluent 2). Combine effluents 1 and 2. If using the LS column, consult the manufacturer's instructions for more information.

j. Centrifuge cells at **300 rcf** for **5 min** (PBMCs) or **10 min** (dissociated tumor cells).

k. Remove the supernatant without disturbing the pellet.

l. Using a **wide-bore** pipette tip, add **1 ml** 1X PBS + 0.04 % BSA to each tube and gently pipette mix 5x to resuspend cell pellet.

m. Transfer the cell suspension to a 2-ml tube.

n. Centrifuge cells at **300 rcf** for **5 min** (PBMCs) or **10 min** (dissociated tumor cells).

o. Remove the supernatant without disturbing the pellet.

p. Resuspend pellet in **1 ml** 1X PBS + 0.04 %.

q. **Repeat** n-o for a total of two washes.

r. Resuspend pellet in 1X PBS + 0.04 % BSA using a **regular-bore** pipette tip to achieve a cell concentration of 700-1,200 cells/µl. Gently pipette mix 10x or until cells are completely suspended.

s. Determine the cell concentration using a Countess II FL Automated Cell Counter. If necessary, dilute the cells with additional 1X PBS + 0.04% BSA until the target cell concentration is reached.

t. Once the target cell concentration is achieved, place the cells on ice.

u. Proceed **immediately** to the 10x Genomics Single Cell protocols.

Results

To demonstrate the efficiency of this protocol, a PBMC sample containing a high percentage of non-viable or dead cells was used. The results obtained following this protocol are outlined below. To determine percent viability, cells were stained with trypan blue and counted using a Countess II FL Automated Cell Counter.

Sample	Total Cells Before Removal	% Live Before Removal	% Live After Removal
PBMCs	1.0 x 10^6	49%	85%
Colorectal cancer	6.5 x 10^6	55%	80%
Clear cell renal carcinoma	3.7 x 10^6	30%	70%

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